Evaluation of intraocular pressure in conscious Hermann’s tortoises (Testudo hermanni) by means of rebound tonometry

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Objective—To determine intraocular pressure (IOP) in healthy Hermann’s tortoises (Testudo hermanni).

Animals—26 outdoor-housed Hermann’s tortoises (13 males and 13 females); body weight ranged from 255 to 2,310 g, and age ranged from 4 to > 50 years.

Procedures—After a preliminary ophthalmic evaluation was performed, IOP was measured by means of a rebound tonometer in both eyes of each tortoise. Three measurements were obtained for each eye; successive measurements were obtained from alternate eyes. Each measurement was based on the mean of 6 values automatically provided by the rebound tonometer. Statistical analysis was used to evaluate correlations between variables and to identify sex- or size-related IOP variations, and changes in IOP over multiple measurements.

Results—Mean ± SEM IOP of the 52 eyes was 15.74 ± 0.20 mm Hg (range, 9 to 22 mm Hg). Results for t tests did not reveal significant differences in IOP between the right and left eyes or between males and females. A significant moderate negative correlation (r = –0.41; r² = 0.169) between IOP and body weight was detected. Results of repeated-measures ANOVA revealed a significant increase in IOP over multiple measurements.

Conclusions and Clinical Relevance—Rebound tonometry was a practical and rapid means of determining IOP in small- to medium-sized tortoises that required minimal manual restraint of the animals. Establishing IOP values in healthy Hermann’s tortoises will provide a reference frame for use during complete ophthalmic examinations, thus allowing clinicians to diagnose a broader spectrum of ocular pathological conditions in tortoises. (Am J Vet Res 2012;73:1807–1812)

Ocular pathological conditions are frequently reported in tortoises.1,2 Specific reference ranges for ocular variables are needed to be able to perform a thorough ophthalmic evaluation of reptile patients. The assessment of IOP is crucial for a complete ophthalmic examination because IOP contributes to the diagnosis of severe ocular diseases, such as glaucoma or uveitis.3 Glaucoma has been described in domestic animals4 but only rarely in wild or exotic species.5–10 To our knowledge, there have been no reports of glaucoma in reptiles. The scarcity of findings likely stems from the problems associated with providing veterinary care for wildlife and exotic animals as well as from the lack of reference intervals for IOP in most exotic and wildlife species.11

Intraocular pressure can be indirectly measured by means of tonometry via indentation,12,13 applanation,14–17 or rebound techniques.18 To our knowledge, IOP has been determined in 4 reptile species: American alligators (Alligator mississippiensis),19 loggerhead sea turtles (Caretta caretta),20 red-footed tortoises (Geochelone carbonaria),21 and yellow-footed tortoises (Geochelone denticulata).22 Previous studies conducted on reptiles have been based exclusively on results for applanation tonometers. Rebound tonometry, a recently developed technique,23 is suited to the measurement of IOP in species with small ocular globes.24–26 In addition, because there is only light, short-lasting contact with the cornea during rebound tonometry, this technique obviates the need for topical anesthesia.23

Hermann’s tortoise (Testudo hermanni) is one of the most popular reptiles kept as pets in Europe. The small size (carapace rarely exceeds 210 mm in length in wild populations),27 herbivorous habits, and toughness make these tortoises an exceptional garden inhabitant.
Although Hermann’s tortoise is listed as a near-threatened species, the number of such tortoises in the wild continues to decrease. This species is protected by the Bern Convention and by the European Habitats Directive, while international trade of this species is regulated by the Washington Convention. The objective of the study reported here was to determine IOP in unanesthetized, healthy Hermann’s tortoises.

Materials and Methods

Animals—The study population consisted of 26 client-owned Hermann’s tortoises ranging in body weight from 235 to 2,310 g and in age from 4 to > 50 years. The tortoises were brought to the Clinica per Animali Esotici for their annual health examination prior to hibernation. Only tortoises kept outdoors and living in seminatural conditions were included in the study. Sex of the tortoises (13 males and 13 females) was ascertained by measuring the tail length and plastron concavity; all tortoises were unequivocally sexed on the basis of those 2 variables, so there was no need to evaluate other dimorphic characteristics. The owners gave informed consent for the participation of their tortoises in the study. The study was performed in compliance with the European Parliament and Council Directive 2010/63/EU.

Procedures—A clinical examination, including ascertainment of age and body weight, was performed on each tortoise. Slit-lamp biomicroscopy and indirect ophthalmoscopy with a 90-diopter lens did not reveal any abnormalities. After the preliminary ophthalmic evaluation, IOP was measured in each eye by means of rebound tonometry. The rebound tonometer did not have an internal calibration table that was not specific for any species; a calibration table obtained from the manufacturer was used. A 1-way repeated-measures ANOVA was used to test for any systematic pattern over the 3 IOP measurements for each eye, followed by the Tukey honestly significant difference post hoc comparison. A 1-way ANOVA was used to test for any iatrogenic changes in IOP, and a 1-way ANOVA was used to test for any systematic pattern over the 3 IOP measurements for each eye. The instrument-generated mean value and any unusual values for either eye were discarded when an erroneous IOP measurement resulted from movement of a tortoise’s head or when the tonometer probe did not come into contact with the central cornea. Seven tortoises were monitored via periodic ophthalmic examinations for 4 weeks after IOP measurements were obtained.

Statistical analysis—Data were analyzed with commercial software. The mean and SEM of the 3 separate IOP measurements were calculated for each eye. Data were evaluated for normality by means of the Shapiro-Wilk test. The mean IOPs of the right eye and left eye were considered as the basic IOP values in each tortoise. The 95% CI of the IOP for each tortoise, the right eye, the left eye, and the 3 measurements of each eye were calculated. The Pearson product moment correlation coefficient was used to assess associations between the IOP of the right eye and left eye and between body weight and IOP of each tortoise. Values outside the range of ± 1.5 SDs were considered outliers. A paired t test was used to assess bias between the IOPs of the right and left eyes. An independent sample t test was used to compare the mean of the IOPs between males and females. ANCOVA was used to examine the effect of body weight and sex on IOP in the sample population. To ascertain whether repeated IOP measurements may have induced iatrogenic changes in IOP, a 1-way repeated-measures ANOVA was used to test for any systematic pattern over the 3 IOP measurements for each eye, followed by the Tukey honestly significant difference post hoc comparison. A 1-way ANOVA for unpaired samples was used to compare the mean IOP for each tortoise obtained in the present study with the mean IOP for each tortoise in previous studies.

Results

Data were normally distributed. Mean ± SEM IOP of the 52 eyes was 15.74 ± 0.20 mm Hg (range, 9 to 22 mm Hg; 95% CI, 15.21 to 16.27 mm Hg). Values were reported as mean ± SEM and range, unless otherwise specified. Two-sided values of P < 0.05 were considered significant.

Table 1—Mean IOP in 13 male and 13 female Hermann’s tortoises (Testudo hermanni).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Mean weight (g)*</th>
<th>Measured†</th>
<th>Adjusted‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>567.2</td>
<td>16.00</td>
<td>15.75</td>
</tr>
<tr>
<td>Female</td>
<td>1,257.0</td>
<td>15.50</td>
<td>15.75</td>
</tr>
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*Male and female mean weight was evaluated as a concomitant variable. †Male and female mean IOP was evaluated as a dependent variable. ‡Adjusted in an ANCOVA on the basis of body weight.

Figure 1—Graph of IOP and body weight in 26 Hermann’s tortoises (Testudo hermanni). Each symbol represents results for 1 tortoise. Observations outside the range of ± 1.5 SDs were considered outliers. There was a significant moderate negative correlation (r = −0.41; P = 0.041) between IOP and body weight.
Intraocular pressure in reptiles has previously been evaluated only by means of applanation tonometry.19–22 Our research group has attempted to measure IOP in Hermann’s tortoises via applanation tonometry, but we were unsuccessful because of the excessive manual restraint required. Rebound tonometry proved to be both faster and more feasible in view of the relatively small size of the ocular globe of these tortoises. Ever since rebound tonometry was first developed,23,39 this instrument has allowed IOP to be measured in small animals (eg, rats78 and mice79), thereby avoiding the need for general anesthesia77 and the effects of anesthesia on IOP.80 Rebound techniques have been reported for horses,33,36,38 koi fish (Cyprinus carpio),34 Eurasian eagle owls (Bubo bubo),40 pigmy goats (Capra hircus),41 Eastern screech owls (Megascops asio),42 rhesus monkeys (Macaca mulatta),43 cats,44 chinchillas (Chinchilla laniger),45 birds of prey,46 Tibetan monkeys (Macaca thibetana),47 rabbits,48 and pigs.49 Moreover, rebound tonometry has proved to be more practical than applanation tonometry in various species.50,51

The amount of agreement between the rebound and applanation tonometry methods has been assessed in a number of species. In humans, the correlation between these 2 IOP measurement methods is good, even at extreme IOPs. Furthermore, rebound tonometry is more practical than is applanation tonometry because rebound tonometry obviates the need for topical anesthesia.51 Rebound tonometry was also strongly correlated with direct manualometry when used to measure the IOP in enucleated eyes of dogs and horses.33 In rabbits, the correlation between the values yielded by rebound and applanation tonometry was weak ($r^2 = 0.357$), with the rebound tonometer proving to be more accurate than the applanation tonometer as a means of measuring IOP.52 In birds, the values yielded by these 2 instruments differ significantly, although the correlation between the rebound and applanation measurements is moderate ($r^2 = 0.55$).54 Values obtained in guinea pigs also differ significantly, and the amount of disagreement between these 2 methods is high.50 In addition, investigators in 1 study53 reported that use of drops of a local anesthetic (oxybuprocaine and betoxycaine) altered IOP in humans. In view of the agreement between the pressure values likely to yield inaccurate results. By giving the need to reduce manual restraint to prevent possible artifacts caused by an increase in IOP caused by pressure on the jugular veins,54,55 we recommend the use of rebound tonometry to measure IOP in small- to medium-sized tortoises.

In reptiles, there are marked variations in IOP between species,19–21 which makes extrapolation of pressure values likely to yield inaccurate results. By contrast, IOP in terrestrial chelonians appears to vary less, with values ranging from a mean $\pm$ SEM of 14.2 $\pm$ 1.2 mm Hg in yellow-footed tortoises22 to a mean $\pm$ SD of 15.3 $\pm$ 8.81 mm Hg in red-footed tortoises22 and a mean $\pm$ SEM of 15.74 $\pm$ 0.20 mm Hg in Hermann’s tortoises. In the study reported here, an ANOVA was used to test the null hypothesis that different species of tortoises would have a similar IOP. We failed to reject the null hypothesis ($P = 0.329$), which indicated that the tortoises had a similar IOP. However, caution should be used when comparing the findings of studies that involved the use of different tonometry techniques.

### Table 2—The IOP (mm Hg) in the right and left eyes of 26 Hermann’s tortoises and effects of multiple measurements on IOP values.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of eyes</th>
<th>Mean $\pm$ SEM</th>
<th>Range</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right eye</td>
<td>26</td>
<td>15.94 $\pm$ 0.44</td>
<td>9–22</td>
<td>15.03–16.85</td>
</tr>
<tr>
<td>Left eye</td>
<td>26</td>
<td>15.55 $\pm$ 0.30</td>
<td>9–21</td>
<td>14.93–16.17</td>
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Measurement*  
First | 52          | 15.13 $\pm$ 0.33 | 10–20 | 14.45–15.80 |
Second | 52          | 15.86 $\pm$ 0.35 | 9–21  | 15.14–16.58 |
Third | 52          | 16.25 $\pm$ 0.39 | 9–22  | 15.51–16.98 |

*Mean values differed significantly ($P = 0.020$; ANOVA) among the 3 measurements.

Figure 2—Box-and-whiskers plots of IOP over 3 measurements in 26 Hermann’s tortoises. The IOPs for the right and left eye of each turtle were combined for each of the 3 measurements. For each measurement, the box represents the median, and the whiskers represent the range. *Value differs significantly ($P < 0.05$) from the value for the first measurement.

IOP for the 26 left eyes was 15.94 $\pm$ 0.30 mm Hg (range, 9 to 21 mm Hg). Mean $\pm$ SEM IOP for the 26 right eyes was 15.94 $\pm$ 0.44 mm Hg (range, 9 to 22 mm Hg). A moderate negative correlation ($r = 0.435$; $r^2 = 0.194$; $P < 0.001$) was detected for the IOP between the right and left eyes. A moderate negative correlation ($r = 0.41$; $r^2 = 0.169$; $P = 0.04$) was detected between IOP and body weight (Figure 1). No significant differences in IOP between males and females was detected when an unpaired t test was used ($P = 0.466$) or when weight was considered as a dependent variable ($P = 0.99$; Table 1). Results for the paired t test did not reveal significant differences ($P = 0.356$) between IOP over the 3 measurements (Table 2). The post hoc test detected significant differences between the first and third measurements (Figure 2).

No evidence of ocular changes was observed in the 7 tortoises during the 4 weeks after tonometry. Results of an unpaired-samples ANOVA did not reveal significant differences ($P = 0.329$) when the results of the present study were compared with those of previous studies.

### Discussion

Intraocular pressure in reptiles has previously been evaluated only by means of applanation tonometry.19–22
Intraocular pressure has been measured in tropical, nonhibernating terrestrial chelonians.\textsuperscript{21,22} In the present study, we decided to evaluate IOP in Hermann's tortoises because this species alternates periods of hibernation (November through March or April) and activity (March or April through October).\textsuperscript{17} The IOP values in hibernating species are of considerable interest because chelonians appear to be extremely susceptible to keratopathies, uveitis, and cataracts after exposure to freezing temperatures.\textsuperscript{38} These pathological conditions predispose mammals to secondary glaucoma.\textsuperscript{3} Although this pathogenic process has not been reported in reptiles, appropriate reference ranges would make it possible to investigate IOP changes in chelonians after hibernation.

The tortoises in the present study were maintained in physiologic ventral recumbency during examination. Recumbency-related IOP variations were observed in 1 study\textsuperscript{38} conducted on loggerhead sea turtles, in which the mean IOP was 23 mm Hg when turtles were in the suspended head-down position but only 5 and 7 mm Hg when in dorsal and ventral recumbency, respectively. Moreover, 4 turtles suspended in the head-down position developed edema of the periccular tissues. The effect of body position on IOP has also been evaluated in humans,\textsuperscript{39,60} bats (hanging and upright positions),\textsuperscript{39} and horses (with the head positioned below and above the level of the heart).\textsuperscript{61} In all these studies, IOP was higher for the head-down positions. In the present study, similar to results for previous studies\textsuperscript{19–22} performed on terrestrial chelonians, variations in IOP attributable to body position were not investigated. Consequently, we strongly recommend that IOP be measured on animals positioned in ventral recumbency until details on posture-related changes in IOP of terrestrial chelonians become available.

No sex-related differences in IOP have been detected in previous studies conducted on reptiles,\textsuperscript{19–22} birds,\textsuperscript{45} and nonconventional mammals,\textsuperscript{41,30,62–64} except for lions (\textit{Panthera leo}).\textsuperscript{46} No sex-related differences were detected in the present study; even after means were adjusted in the ANCOVA with weight as a covariate (15.75 mm Hg vs 15.75 mm Hg; \(P = 0.99\)).

A weak negative correlation was detected between tortoise body weight and IOP. A similar observation has been made in alligators\textsuperscript{47} (in which IOP is negatively correlated with increasing body length) as well as llamas and alpacas\textsuperscript{48} (in which IOP decreases with increasing age).

Circadian rhythms in IOP have been described in humans,\textsuperscript{46,67} and domestic animals, including dogs,\textsuperscript{68–69} cats,\textsuperscript{70} and rabbits.\textsuperscript{71} In nocturnal species such as rabbits, IOP peaks during the night, whereas in diurnal species such as dogs, IOP peaks during the day.\textsuperscript{69–71} Because IOP measurements in the present study were obtained at a relatively constant time of day, circadian variations in IOP were not investigated. Further studies are warranted to investigate circadian rhythms in IOP of tortoises by means of 24-hour IOP monitoring.

Two differences between previous rebound tonometry studies and the study reported here are worthy of mention. First, contact between the cornea and probe, which consists of a gentle and rapid (0.3 m/s) impact on the eye, often does not cause a corneal reflex in humans and dogs.\textsuperscript{21,30,72} By contrast, the impact of the probe invariably induced a corneal reflex in the tortoises in the present study. Although this discrepancy may have been attributable to the size of the ocular globe of the tortoises, interspecies differences in corneal innervation cannot be excluded.

The second unexpected finding was the significant difference in IOP among measurements, with an increase between the first and last measurements. This finding has not been reported in studies based on multiple tonometry measurements in reptiles\textsuperscript{19,21,22} or mammals.\textsuperscript{11,73–76} Some authors have suggested that the tonographic effect of an aqueous massage is negligible for a rebound tonometer because contact with the eye is extremely slight when this technique is used.\textsuperscript{38,77} By contrast, reports of a decrease in IOP after rebound tonometry do exist, with a marked reduction in IOP observed in mice undergoing multiple rebound tonometry measurements.\textsuperscript{78} No significant changes in IOP were observed in healthy humans undergoing successive applanation tonometry, whereas in glaucomatous patients, the decrease between the first and second IOP measurements was significant, as was the decrease in IOP between the first measurement and each subsequent measurement.\textsuperscript{79} In another study,\textsuperscript{80} investigators found that series of measurements with a rebound tonometer had a tonographic effect (decrease in IOP from 14.6 mm Hg at the first measurement to 14.2 mm Hg at the second to 14.0 mm Hg at the third measurement [total decrease of 0.6 mm Hg after a series of 18 successive measurements]).

We believe that the increase in IOP detected in the present study can be ascribed to the peculiar visual accommodation mechanism of chelonians. Accommodation occurs by altering the distance between the cornea and fundus. The pressure exerted by the ciliary body, which is mediated by the ciliary muscle, against the lens equator increases the anteroposterior diameter of the lens.\textsuperscript{78,79} We suspect that the repeated stimulus caused by multiple measurements resulted in the tortoises trying to focus on the probe; the resulting accommodation-induced change in the shape of the eye led to an increase in IOP during subsequent measurements. Further studies are warranted to confirm whether such changes in IOP can be caused by accommodation. Although the increase in IOP was small (1.12 mm Hg [from 15.13 mm Hg for the first measurement to 16.25 mm Hg for the third measurement]), the possible clinical consequences of rebound tonometry in tortoises, especially in tortoises with ocular pathological conditions, need to be investigated further.

The present study provided information needed to perform a tonometric evaluation in Hermann's tortoises. To the authors' knowledge, this is the first report of IOP evaluation in a hibernating tortoise. Because the rebound tonometer used did not have a calibration table for reptiles, we applied a calibration table for nonspecific species. Considering the peculiar anatomy of the eyes of chelonians (ie, sclera ossicles, a thick scleral cartilage cup, and an avascular retina),\textsuperscript{40} further studies designed to compare rebound tonometry measurements with manometric measurements are warranted.
to establish the accuracy of rebound tonometry in Hermann's tortoises.

References

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